(12) UK Patent Application (19) GB (11) 2 331 750 (13) A

(43) Date of A Publication 02.06.1999

(21) Application No 9806989.1

(22) Date of Filing 02.04.1998

(30) Priority Data
(31) 09343630 (32) 01.12.1997 (33) JP

(51) INT CL[6]
C12N 15/56 // (C12N 15/56 C12R 1:465)

(52) UK CL (Edition Q)
C3H HB1 HB7E H650 H732
C6Y YG11 Y183

(56) Documents Cited
WO 91/06009 A1

(58) Field of Search
INT CL[6] C12N 15/56
Online: CAS ONLINE, DGENE, DIALOG/BIOTECH, WPI

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(54) Abstract Title
Gene for a cell wall lytic enzyme

(57) The genes for a cell wall lytic enzyme from Streptomyces, and a precursor thereof have been isolated (Nucleotide Sequences Nos 2 and 1 herein). The genes have been incorporated into plasmids and the plasmids used to transform an E. coli (FERM BP-6166) expression of which makes the enzyme available on an industrial scale. The enzyme is useful in the food industry.

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TITLE

Gene for a Cell Wall Lytic Enzyme

DESCRIPTION

FIELD OF THE INVENTION

The invention relates to a gene of a cell wall lytic enzyme, a plasmid vector containing said gene and a transformant.

BACKGROUND OF THE INVENTION

Cell wall lytic enzymes are enzymes which degrade the cell walls of bacteria including Actinomycetes. On the action of the enzyme, the bacterial cell wall is decomposed, leading to the death of the bacteria.

The outer layer of bacteria is covered with cell wall, and the principal structural component of the cell wall is a peptide glycan comprising sugar chains and peptides. The cell wall lytic enzyme acts on the peptide glycan.

When the enzyme acts on the peptide glycan, the enzyme reacts with the sugar chain of the peptide glycan to generate N-acetylmuramic acid from the sugar at the terminus to be reduced. Therefore, the enzyme is classified as N-acetylmuramidase.

Major enzymes to be classified as N-acetylmuramidase include lysozyme derived from chicken egg white. However, the cell wall lytic enzyme is different from these enzymes in terms of enzymatic and chemical properties and the subjective microorganisms to be decomposed [Hayashi K., et al., Agric. Biol. Chem. (European Edition of Japanese Journal of Agriculture, Biochemistry and Chemistry), Vol. 45, pp. 2289-2300, 1981], and the enzyme has novel specificities.

As has been described above, the cell wall lytic enzyme decomposes bacterial cell wall, and by utilizing the property, the enzyme is used for extracting enzymes and DNA present in the inside of bacteria.

Because some bacteria may be killed through the action of the present enzyme, the enzyme may be utilized

as a food preservative [Hayashi K., et al., Agric. Biol. Chem. (European Edition of Japanese Journal of Agriculture, Biochemistry and Chemistry), Vol. 53, pp. 3173-3177, 1989].

Conventional methods for recovering the cell wall lytic enzyme include a method comprising culturing microorganisms, such as Actinomycetes belonging to genus Streptomyces, and bacteria belonging to genera Achromobacter, Aeromonas, Bacillus, Clostridium, Flavobacterium, Myxobacter, Myxococcus, Pseudomonas, Staphylococcus and Streptococcus, and preparing the objective enzyme from the culture filtrate or the cultured bacteria. When the cell wall lytic enzyme is egg white-derived lysozyme, use is made of a method comprising preparing the enzyme by utilizing isoelectric precipitation and the like.

The enzyme recovered by these methods is commercially available as crude enzyme or purified enzyme. However, these methods are not satisfactory as methods for producing the enzyme in a stable fashion.

By cloning the gene of the enzymes to elucidate their structure and expressing the gene, the invention contributes to the industrial production of cell wall lytic enzymes.

SUMMARY OF THE INVENTION

The invention provides the gene of a precusor of a cell wall lytic enzyme derived from a microorganism belonging to the genus Streptomyces, the gene having the nucleotide sequence shown as Sequence No. 1 in the Sequence Listing. The invention also provides a plasmid containing this gene and an E. coli (FERM BP-6166) transformed with the plasmid.

In another embodiment, the invention provides the gene of a cell wall lytic enzyme derived from a microorganism belonging to the genus Streptomyces, the gene having the nucleotide sequence shown as Sequence No. 2 in the Sequence Listing. The invention also provides a

plasmid containing this gene and an E. coli transformed with the plasmid.

DETAILED DESCRIPTION OF THE INVENTION

The inventors extracted a cell wall lytic enzyme from a bacterium having the ability of producing a cell wall lytic enzyme, belonging to the genus Streptomyces. The enzyme was purified to high purity and the amino acid sequence of the N-terminus (see Sequence No. 3 in the Sequence Listing) was determined. On the basis of the amino acid sequence so determined, a pair of primers was prepared (see Sequence Nos. 4 and 5 in the Sequence Listing). By polymerase chain reaction (PCR) with the genomic DNA extracted from a bacterium belonging to the genus Streptomyces as a template by using the primers mentioned above, a prominent band of 140 bp was recovered.

By cloning the resulting band (PCR product) and analyzing the band with a DNA sequencer, the DNA nucleotide sequence thereof was determined (see Sequence No. 6 in the Sequence Listing). The DNA nucleotide sequence was then translated into amino acid. There was observed a sequence corresponding to the preliminarily recovered amino acid sequence at the N-terminus (see Sequence No. 3 in the Sequence Listing), which indicates that said PCR product was part of the gene of the cell wall lytic enzyme.

Then, the gene of the cell wall lytic enzyme was firstly cloned by using the PCR product as the probe.

Alternatively, the genomic DNA extracted from the bacterium belonging to the genus Streptomyces was enzymatically degraded, to subject the resulting DNA fragments to Southern hybridization. Consequently, it was confirmed that the objective gene of the cell wall lytic enzyme was present in the DNA fragment of 2.8 kbp.

By sub-cloning the fragment containing the gene of the cell wall lytic enzyme, a plasmid was prepared. The plasmid was used for transformation into E. coli, to obtain a transformant.

As has been described above, the gene of a cell wall lytic enzyme of the invention is derived from microorganism having an ability of producing a cell wall lytic enzyme. Such a microorganism may, for example, be Actinomycetes of genus Streptomyces, and bacteria belonging to genera Achromobacter, Aeromonas, Bacillus, Clostridium, Flavobacterium, Myxobacter, Myxococcus, Pseudomonas, Staphylococcus, Streptococcus and the like.

Among them, preferably, use is made of bacteria belonging to genus Streptomyces. The bacterial strains belonging to genus Streptomyces include for example Streptomyces rutgersensis H-46.

The cell wall lytic enzyme can be recovered from the aforementioned microorganisms. More specifically, the aforementioned bacterial strains are cultured by routine methods. The culture medium is preferably a medium containing defatted soy bean extract, but is not limited thereto. Cultivation can be carried out for example by the method of Hayashi K., et al., J. Ferment. Technol. (European Edition of Japanese Journal of Fermentation Engineering Association), Vol. 59, pp. 319-323, 1981.

The culture broth is centrifuged to remove the microorganism. From the supernatant thus obtained, a highly purified cell wall lytic enzyme can be recovered by routine purification means, such as ion exchange chromatography, column chromatography, FPLC, HPLC, etc.

One example of such purification means is the method of Hayashi K., et al., Agric. Biol. Chem, (European Edition of Japanese Journal of Agriculture, Biochemistry and Chemistry), Vol. 45, pp. 2289-2300, 1981. More specifically, the enzyme can be purified by column chromatography on cation exchange resin.

Then, the amino acid sequence at the N-terminus of the purified cell wall lytic enzyme was determined. For sequencing, a protein sequencer of Type G 1005A (manufactured by Hewlett Packard, Co.) can be used. The determined amino acid sequence at the N-terminus is shown as Sequence No. 3 in the Sequence Listing.

By determining the nucleotide sequence from the determined amino acid sequence and preparing primers (see Sequences Nos. 4 and 5 in the Sequence Listing) prepared on the basis of the nucleotide sequence, PCR was carried out with the genomic DNA extracted from the bacterial strain belonging to genus Streptomyces as a template by using said primers. Consequently, a prominent band of 140 bp was recovered.

So as to analyze the DNA nucleotide sequence of the resulting band, the band was cloned for the analysis with a DNA sequencer. The nucleotide sequence thus recovered by the analysis (see Sequence No. 5 in the Sequence Listing) was then translated into amino acid. A sequence corresponding to the preliminary amino acid sequence at the N-terminus (see Sequence No. 3 in the Sequence Listing) was observed, which indicates that the product recovered by PCR was a part of the gene of the cell wall lytic enzyme.

Then, the gene of the precursor, including the gene of the mature cell wall lytic enzyme, was cloned, by using the PCR product as a probe.

Firstly, genomic DNA is extracted from a bacterium belonging to genus Streptomyces. The extraction can be carried out for example by the method of Saito, "Protein and Nucleic Acid and Enzyme", Vol. 11, pp. 446. More specifically, the cell wall of the bacterium was enzymatically degraded, to wind the extracted DNA over a glass bar, to purify the genomic DNA.

The nucleotide sequence and amino acid sequence of the precursor of the cell wall lytic enzyme in accordance with the invention are shown in Sequence No. 1 in the Sequence Listing. From the amino acid sequence of the precursor gene of said gene of the cell wall lytic enzyme, furthermore, the amino acid sequence of the gene of the cell wall lytic enzyme was constructed on the basis of the preliminary determined amino acid sequence at the N-terminus of the cell wall lytic enzyme (see Sequence No. 3 in the Sequence Listing). The amino acid sequence of

said gene is shown together with the nucleotide sequence thereof in Sequence No. 2 in the Sequence Listing.

The gene of the cell wall lytic enzyme in accordance with the invention is an enzyme having a novel amino acid sequence, and no protein with 55% or higher homology to the enzyme has been found.

By subcloning the 2.8-kbp fragment prepared by agarose gel electrophoresis by using a DNA ligation kit (manufactured by Takara Brewery, Co.) in a plasmid preliminarily dephosphorylated, a plasmid pUC 18-SR1 was prepared.

The plasmid was then transformed into E. coli by routine method. The transformed E. coli as deposited on 17 November 1997 under the Budapest Treaty at the National Institute of Bioscience and Human Technology, Agency of Industrial Science and Technology (1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan). The accession number given to the deposit was FERM BP-6166. Furthermore, the plasmid pUC 18-SR1 contains the gene of the cell wall lytic enzyme.

The expression of the gene of the cell wall lytic enzyme can be confirmed, by culturing the transformant E. coli thus recovered and assaying said E. coli and the cell wall lytic enzyme in the supernatant.

By culturing the transformant in a nutrition medium at 20 to 37°C for 3 to 48 hours and disrupting the resulting microbial strain and purifying the supernatant recovered by separation of the liquid from the solid in accordance with a routine method, the cell wall lytic enzyme can be recovered.

According to the invention, the gene of the enzyme acting on bacterial cell walls to decompose the cell walls is provided. The enzyme recovered through the expression of the gene is useful in the food industry.

EXAMPLE

The invention is illustrated in detail by this Example.

Microorganism Streptomyces rutgersensis H-46 was cultured in a culture medium containing 0.5% glucose and 2% defatted soy bean hot-water extract, following the method of Hayashi K., et al., J. Ferment. Technol. (European Edition of Japanese Journal of Fermentation Engineering Association), Vol. 59, pp. 319-323, 1981.

Using ion exchange chromatography according to the method of Hayashi K., et al., Agric. Biol. Chem. (European Edition of Japanese Journal of Agriculture, Biochemistry and Chemistry), Vol. 45, pp. 2289-2300, 1981, a highly purified cell wall lytic enzyme was recovered from the supernatant obtained by eliminating the microbial strain from the culture broth.

Using the purified enzyme, the amino acid sequence at the N-terminus was determined by a protein sequencer Type G 1005A (manufactured by Hewlett Packard, Co.). The determined sequence is shown as Sequence No. 3 in the Sequence Listing.

From the amino acid sequence determined, two regions with less codon stringency were selected to chemically synthesize a forward primer (shown as Sequence No. 4 in the Sequence Listing) and a reverse primer (shown as Sequence No. 5 in the Sequence Listing).

Using these primers, amplification was effected by PCR, with the genomic DNA of the strain H-46 of Streptomyces rutgersensis as a template. Consequently, a prominent band of 140 bp was recovered.

By cloning the resulting band and analyzing the band with a DNA sequencer, the DNA nucleotide sequence thereof was determined as shown in Sequence No. 6 in the Sequence Listing. The DNA nucleotide sequence was then translated into amino acid. There was observed a sequence corresponding to the preliminarily recovered amino acid sequence at the N-terminus as shown as Sequence No. 3 in the Sequence Listing.

It is thus indicated that the PCR product was a part of the gene of the cell wall lytic enzyme.

Then, the PCR product was labelled with Gene Image Chemiluminescence Nucleic Acid Detection System (manufactured by Amersham, Co.), and by using the labelled product as the probe, the gene of the precursor of the cell wall lytic enzyme was cloned.

Alternatively, the genomic DNA was extracted from Streptomyces rutgersensis H-46 by the method of Saito: "Protein, Nucleic Acid and Enzyme", Vol. 11, pp.446. The genomic DNA was then completely decomposed with a restriction enzyme Sac I. The resulting restriction cleavage products were separated by agarose gel electrophoresis, and then subjected to Southern hybridization ("Cloning and Sequence", Watanabe eds, Noson Bunka-sha, 1989, pp 157). Consequently, it was confirmed that the objective gene of the cell wall lytic enzyme was present in the DNA fragment of 2.8 kbp.

The 2.8 kbp fragment was prepared by agarose gel electrophoresis, according to the method described in Sambrook, J., Fritsch, E.F. and Maniatis, T., "Molecular Cloning; A Laboratory Manual, 2nd edition", Section 6.3, Vol. 1 (1989).

Alternatively, the plasmid pUS-18 was cleaved with a restriction enzyme Sac I, followed by dephosphorylation with alkali phosphatase. The 2.8 kbp fragment was sub-cloned into the dephosphorylated plasmid by using a DNA ligation kit (manufactured by Takara Brewery, Co.) by the method described in Cloning and Sequence, Watanabe eds, Noson Bunka-sha, 1989, pp. 134, to prepare a plasmid pUC 18-SR1.

The plasmid was transformed into E. coli, according to the method described in Sambrook, J., Fritsch. E.F. and Maniatis, T., "Molecular Cloning; A Laboratory Manual, second edition", Section 1.74, Vol. 1 (1989). Furthermore, the plasmid pUC 18-SR1 contains the gene of the cell wall lytic enzyme. The transformant according to claim 10 can also be obtained by the same method.

A greater volume of the plasmid pUS 18-SR1 was prepared from the transformant, for analysis with

d-Rhodamine-Terminator Cycle Sequencing Kit (manufactured by Perkin Elmer, Co.).

Linking the information of the determined nucleotide sequence together, the gene of the precursor of the cell wall lytic enzyme was constructed. The nucleotide sequence and amino acid sequence of said gene of the precursor are shown as Sequence No. 1 in the Sequence Listing.

The amino acid sequence of the precursor gene of the cell wall lytic enzyme, as shown as Sequence No.1 in the Sequence Listing, is compared with the preliminarily recovered amino acid sequence of the N-terminus of the cell wall lytic enzyme (see Sequence No.3 in the Sequence Listing).

Consequently, the amino acid sequence of the N-terminus of the cell wall lytic enzyme (see Sequence No.3 in the Sequence Listing) agrees with the sequence from the 21st residue to 100th residue in the amino acid sequence as shown in Sequence No.1. It is thus indicated that the gene of the cell wall lytic enzyme can be found downstream the 241st residue of the nucleotide sequence of the precursor gene. The gene of the active cell wall lytic enzyme was constructed from the precursor gene of the cell wall lytic enzyme, on the basis of the amino acid sequence of the N-terminus of the cell wall lytic enzyme, which is shown in Sequence No.2 in the Sequence Listing.

The molecular weight of the active cell wall lytic enzyme was determined by a laser ionization system Type TOF-MS KOMPACT MALDI III manufactured by Shimadzu, Co. Ltd.. The molecular weight was 23,000 daltons, which agrees well with the molecular weight of the protein encoded by the present gene, i.e. 23,056 daltons.

SEQUENCE LISTING

Sequence No.: 1

Sequence Length : 1088

Type of Sequence : nucleic acid

Strandedness : double-stranded

Topology : linear

Molecular Type of Sequence : genomic DNA

Origin:

Name of Organism : Streptomyces rutgersensis

Name of Strain : H-46

Direct Origin:

Name of Plasmid : pUC 18-SR1

Sequence Characteristics:

Symbol Representing the Characteristics : CDS

Location : 181..870

Method for Determining the Characteristics : P

Sequence:

```
TCAGGCACCC CCCGTCACGC TCGCCCACCG CCTTCGGAGG CCCCCATGCG CGTACCCAGA   60
TCCGGAGCCC GCCCCTCTCG CCGCACCGCG GCCGGAGTTC TCCTCGCCGC CCTCTCCCTG  120
CTCTTCACCC TGCCCTCGGG GGCGCACGCC GCCGACCGTC CCGAGCGGGG CGAGGCCCAC  180
ATG GGC ATG GGC GTC GTG GAG CAC GAC GGC CGG AGC GGG GCG CCC GGT     228
Met Gly Met Gly Val Val Glu His Asp Gly Arg Ser Gly Ala Pro Gly
                5                  10                  15
ATC TCG CCG CGC GCC GTG CAG ACG GAG GGC GTG GAC GTC TCC AGC CAT     276
Ile Ser Pro Arg Ala Val Gln Thr Glu Gly Val Asp Val Ser Ser His
            20                  25                  30
CAG GGG AAC GTC GAC TGG GCC GCG CTG TGG AAC AGC GGC GTC AAG TGG     324
Gln Gly Asn Val Asp Trp Ala Ala Leu Trp Asn Ser Gly Val Lys Trp
        35                  40                  45
TCG TAC GTG AAG GCC ACC GAG GGC ACG TAC TAC AAG AAC CCG TAC TTC     372
Ser Tyr Val Lys Ala Thr Glu Gly Thr Tyr Tyr Lys Asn Pro Tyr Phe
    50                  55                  60
GCG CAG CAG TAC AAC GGC AGT TAC AAC GTG GGG ATG ATC CGC GGC GCC     420
Ala Gln Gln Tyr Asn Gly Ser Tyr Asn Val Gly Met Ile Arg Gly Ala
65                  70                  75                  80
TAC CAC TTC GCG ACG CCC AAC ACG ACG AGC GGC GCC GCC CAG GCC AAC     468
Tyr His Phe Ala Thr Pro Asn Thr Thr Ser Gly Ala Ala Gln Ala Asn
                85                  90                  95
TAC TTC GTG GAC AAC GGC GGC GGC TGG TCC CGC GAC GGC AAG ACC CTG     516
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Tyr Phe Val Asp Asn Gly Gly Gly Trp Ser Arg Asp Gly Lys Thr Leu
                100                 105                 110
CCG GGT GTC CTG GAC ATC GAG TGG AAC CCG TAC GGC GAC CAG TGC TAC   564
Pro Gly Val Leu Asp Ile Glu Trp Asn Pro Tyr Gly Asp Gln Cys Tyr
        115                 120                 125
GGC CTG AGC CAG TCC GCG ATG GTC AAC TGG ATC CGC GAC TTC ACC AAC   612
Gly Leu Ser Gln Ser Ala Met Val Asn Trp Ile Arg Asp Phe Thr Asn
    130                 135                 140
ACC TAC AAG GCC CGC ACC GGC CGG GAC GCG GTC ATC TAC ACC GCG ACC   660
Thr Tyr Lys Ala Arg Thr Gly Arg Asp Ala Val Ile Tyr Thr Ala Thr
145                 150                 155                 160
AGC TGG TGG ACC TCC TGC ACC GGC AAC TAC GCG GGC TTC GGC ACC ACC   708
Ser Trp Trp Thr Ser Cys Thr Gly Asn Tyr Ala Gly Phe Gly Thr Thr
                165                 170                 175
AAC CCG CTC TGG GTC GCC CGG TAC GCC GCC TCG GTG GGC GAA CTC CCG   756
Asn Pro Leu Trp Val Ala Arg Tyr Ala Ala Ser Val Gly Glu Leu Pro
            180                 185                 190
GCC GGC TGG GGC TTC TAC ACG ATG TGG CAG TAC ACC TCC ACC GGC CCG   804
Ala Gly Trp Gly Phe Tyr Thr Met Trp Gln Tyr Thr Ser Thr Gly Pro
        195                 200                 205
ATC GTC GGC GAC CAC AAC CGC TTC AAC GGC GCG TAC GAC CGG CTC CAG   852
Ile Val Gly Asp His Asn Arg Phe Asn Gly Ala Tyr Asp Arg Leu Gln
    210                 215                 220
GCG CTC GCC AAC GGC TGAGCCCGAG CCGTCGGACG CCCCGGCGAC CGCGCACGCC   907
Ala Leu Ala Asn Gly
225
GAAGAGGCCC GGTGACCTGT TCACCGGGCC TTTTCCGGGT CCGGAGCGGG GTGCGGAAAT  967
CCTTCCGGGG GCGGGGCAAC CGTTCGACTA TCCACTCCAT CTATACACGG CGTGAACACT 1027
CTGACGCACG CCGAGCCCCG CACCCGCCGC CGCCCGCACC GCATCCGCCG TACAGCCGTC 1087
G                                                                 1088
```

Sequence No. : 2

Length of Sequence : 630

Type of Sequence : nucleic acid

Strandedness : double-stranded

Topology : linear

Molecular Type of Sequence : Genomic DNA

Origin:

Name of Organism : Streptomyces rutgersensis

Name of Strain : H-46

Direct origin:

Name of Plasmid : pUC 18-SR1

Sequence Characteristics:

Symbol Representing the Characteristics : mat peptide

Location : 1..630

Method for Determining the Characteristics : P

Sequence:

```
GCC GTG CAG ACG GAG GGC GTG GAC GTC TCC AGC CAT CAG GGG AAC GTC     48
Ala Val Gln Thr Glu Gly Val Asp Val Ser Ser His Gln Gly Asn Val
                 5                  10                  15

GAC TGG GCC GCG CTG TGG AAC AGC GGC GTC AAG TGG TCG TAC GTG AAG     96
Asp Trp Ala Ala Leu Trp Asn Ser Gly Val Lys Trp Ser Tyr Val Lys
            20                  25                  30

GCC ACC GAG GGC ACG TAC TAC AAG AAC CCG TAC TTC GCG CAG CAG TAC    144
Ala Thr Glu Gly Thr Tyr Tyr Lys Asn Pro Tyr Phe Ala Gln Gln Tyr
        35                  40                  45

AAC GGC AGT TAC AAC GTG GGG ATG ATC CGC GGC GCC TAC CAC TTC GCG    192
Asn Gly Ser Tyr Asn Val Gly Met Ile Arg Gly Ala Tyr His Phe Ala
    50                  55                  60

ACG CCC AAC ACG ACG AGC GGC GCC GCC CAG GCC AAC TAC TTC GTG GAC    240
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Thr Pro Asn Thr Thr Ser Gly Ala Ala Gln Ala Asn Tyr Phe Val Asp
65                  70                  75                  80
AAC GGC GGC GGC TGG TCC CGC GAC GGC AAG ACC CTG CCG GGT GTC CTG   288
Asn Gly Gly Gly Trp Ser Arg Asp Gly Lys Thr Leu Pro Gly Val Leu
                85                  90                  95
GAC ATC GAG TGG AAC CCG TAC GGC GAC CAG TGC TAC GGC CTG AGC CAG   336
Asp Ile Glu Trp Asn Pro Tyr Gly Asp Gln Cys Tyr Gly Leu Ser Gln
            100                 105                 110
TCC GCG ATG GTC AAC TGG ATC CGC GAC TTC ACC AAC ACC TAC AAG GCC   384
Ser Ala Met Val Asn Trp Ile Arg Asp Phe Thr Asn Thr Tyr Lys Ala
        115                 120                 125
CGC ACC GGC CGG GAC GCG GTC ATC TAC ACC GCG ACC AGC TGG TGG ACC   432
Arg Thr Gly Arg Asp Ala Val Ile Tyr Thr Ala Thr Ser Trp Trp Thr
    130                 135                 140
TCC TGC ACC GGC AAC TAC GCG GGC TTC GGC ACC ACC AAC CCG CTC TGG   480
Ser Cys Thr Gly Asn Tyr Ala Gly Phe Gly Thr Thr Asn Pro Leu Trp
145                 150                 155                 160
GTC GCC CGG TAC GCC GCC TCG GTG GGC GAA CTC CCG GCC GGC TGG GGC   528
Val Ala Arg Tyr Ala Ala Ser Val Gly Glu Leu Pro Ala Gly Trp Gly
                165                 170                 175
TTC TAC ACG ATG TGG CAG TAC ACC TCC ACC GGC CCG ATC GTC GGC GAC   576
Phe Tyr Thr Met Trp Gln Tyr Thr Ser Thr Gly Pro Ile Val Gly Asp
            180                 185                 190
CAC AAC CGC TTC AAC GGC GCG TAC GAC CGG CTC CAG GCG CTC GCC AAC   624
His Asn Arg Phe Asn Gly Ala Tyr Asp Arg Leu Gln Ala Leu Ala Asn
        195                 200                 205
GGC TGA                                                          630
Gly
209
```

Sequence No. : 3

Length of Sequence : 80

Type of Sequence : amino acid

Topology : linear

Molecular Type of Sequence : peptide

Type of Fragment : N-terminal fragment

Origin:

Name of Organism : Streptomyces rutgersensis

Name of Strain : H-46

Direct Origin:

Enzyme produced by Streptomyces rutgersensis

Sequence:

Ala	Val	Gln	Thr	Glu	Gly	Val	Asp	Val	Ser	Ser	His	Gln	Gly	Asn	Val
1				5				10						15	
Asp	Trp	Ala	Ala	Leu	Trp	Asn	Ser	Gly	Val	Lys	Trp	Ser	Tyr	Val	Lys
			20					25					30		
Ala	Thr	Glu	Gly	Thr	Tyr	Tyr	Lys	Asn	Pro	Tyr	Phe	Ala	Gln	Gln	Tyr
		35					40					45			
Asn	Gly	Ser	Tyr	Asn	Val	Gly	Met	Ile	Arg	Gly	Ala	Tyr	His	Phe	Ala
	50					55					60				
Thr	Pro	Asn	Thr	Thr	Ser	Gly	Ala	Ala	Gln	Ala	Asn	Tyr	Phe	Val	Asp
65					70					75					80

Sequence No. : 4

Length of Sequence : 20

Type of Sequence : nucleic acid

Strandedness : single-stranded

Topology : linear

Molecular Type of Sequence : other nucleic acids (prepared from amino acid sequence)

Origin:

Name of Organism : Streptomyces rutgersensis

Name of Strain : H-46

Direct Origin:

Enzyme produced by Streptomyces rutgersensis

Sequence:

CARGGSAAYG TSGAYTGGGC 20

Sequence No. : 5

Length of Sequence : 20

Type of Sequence : nucleic acid

Strandedness : single-stranded

Topology : linear

Molecular Type of Sequence : other nucleic acids (prepared from amino acid sequence)

Origin:

Name of Organism : Streptomyces rutgersensis

Name of Strain : H-46

Direct Origin:

Enzyme produced by Streptomyces rutgersensis

Sequence:

CGGATCATSC CSACRTTRTA 20

Sequence No. : 6

Length of Sequence : 137

Type of Sequence : nucleic acid

Strandedness : single-stranded

Topology : linear

Molecular Type of Sequence : other nucleic acids

Origin:

Name of Organism : Streptomyces rutgersensis

Name of Strain : H-46

Direct Origin:

PCR products

Sequence:

```
CAG GGG AAC GTC GAC TGG GCC GCG CTG TGG AAC AGC GGC GTC AAG TGG     48
Gln Gly Asn Val Asp Trp Ala Ala Leu Trp Asn Ser Gly Val Lys Trp
  1               5                  10                  15
TCG TAC GTG AAG GCC ACC GAG GGC ACG TAC TAC AAG AAC CCG TAC TTC     96
Ser Tyr Val Lys Ala Thr Glu Gly Thr Tyr Tyr Lys Asn Pro Tyr Phe
            20                  25                  30
GCG CAG CAG TAC AAC GGC AGT TAC AAC GTG GGG ATG ATC CG             137
Ala Gln Gln Tyr Asn Gly Ser Tyr Asn Val Gly Met Ile
        35                  40                  45
```

CLAIMS

1. A gene of a precursor of a cell wall lytic enzyme derived from a microorganism belonging to a genus Streptomyces, the gene having the nucleotide sequence shown as Sequence No. 1 in the Sequence Listing.

2. A gene according to claim 1, the gene being derived from a microorganism selected from the group consisting of Actinomycetes of genus Streptomyces, and bacteria belonging to genera Achromobacter, Aeromonas, Bacillus, Clostridium, Flavobacterium, Myxobacter, Myxococcus, Pseudomonas, Staphylococcus and Streptococcus.

3. A gene according to claim 1, the gene being derived from the microorganism Streptomyces rutgersensis H-46.

4. A plasmid containing a gene according to any preceding claim.

5. An E. coli (FERM BP-6166) transformed with a plasmid according to claim 4.

6. A gene of a cell wall lytic enzyme derived from a microorganism belonging to a genus Streptomyces, the gene having the nucleotide sequence shown as Sequence No. 2 in the Sequence Listing.

7. A gene according to claim 6, the gene being derived from a microorganism selected from the group consisting of Actinomycetes of genus Streptomyces, and bacteria belonging to genera Achromobacter, Aeromonas, Bacillus, Clostridium, Flavobacterium, Myxobacter, Myxococcus, Pseudomonas, Staphylococcus and Streptococcus.

8. A gene according to claim 6, the gene being derived from the microorganism Streptomyces rutgersensis H-46.

9. A plasmid containing a gene according to any preceding claim.

10. An *E. coli* transformed with a plasmid according to claim 9.

The Patent Office

Application No:	GB 9806989.1	**Examiner:**	Dr Jon Broughton
Claims searched:	1, 3-6 and 8-10	**Date of search:**	27 August 1998

Patents Act 1977
Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.P):

Int Cl (Ed.6): C12N 15/56

Other: ONLINE: CAS ONLINE, DGENE, DIALOG/BIOTECH, WPI

Documents considered to be relevant:

Category	Identity of document and relevant passage	Relevant to claims
A	WO 91/06009 A1 (AMGEN INC) see whole document.	1, 3-6 and 8-10

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.